November 9, 1949.

Dr. L. Cavalli,
Dept. Genetics,
University of Cambridge,
Cambridge, England.

Dear Dr. Cavalli:

I would like very much to have E. coli 123, and would appreciate your sending it to me. Meanwhile, we have found that another coli strain which was isolated from chickens, and which showed evidence of recombination within substrains, also seems to recombine with K-12 mutants. Thus, very surprisingly, the three sexually competent coli strains so far discovered are part of the same mating system! Like 123, the new W-1113 differs from K-12 in many respects- resistance to T phages, sucrose fermenting... Unfortunately, for unexplained reasons, one can select prototrophs occasionally in a single step from the double mutant I have used. I am sending 4 transfers of some W-1113 derivatives under separate cover.

Concerning the mechanism of Hfr, the medium used was Pennassay broth (formula given in papers) in which the pH does go down ultimately. This may well account for the differences. I have the feeling that a nutritional selection is not very satisfactory for quantitative studies on recombination owing to the fact that fusions occur at indeterminate times after plating. I tried an experiment following Newcombe's Nature paper, to try to establish the time at which the recombinant prototrophs begin to proliferate. There was a considerable variance, but respreading the minimal plates after as much as 5 hours gave no augmentation of the number of prototrophs which ultimately came up. I should repeat the experiment with longer time intervals. Rather than use the nutritional selection, I would carry out crosses in liquid medium in which the concentrations of the reacting cells are under some control. I have recently finished some work on the application of drug resistance to the selection of recombinants (started about 3 years ago), and finally found streptomycin x azide to be a satisfactory though not an optimal combination. Streptomycin is a very excellent selector, owing to the complete inhibition of sensitives, and the very low spontaneous mutation rate to resistance (10^{-10}). Azide is usable but not completely satisfactory. In an experiment using 58-161/Az x 677/sr 93/100 of the double resistants selected were recombinations for fermentative, nutritional or phage reactions. The remaining 7 were presumably mostly or all mutations. The azide was used in M/500 concentration. It should be used in a buffered medium to prevent loss of HN_3 in acid, or lack of penetration in alkaline media. Streptomycin was used at 100u/ml. The advantage of this system is that mixing the parents in the plate gives very poor yields compared to growing them together. I think I have already mentioned an impression that Mg is essential for recombination. Bernard D. Davis tells me that he has gotten very poor yields by mixing parents on his minimal medium which

contains substantial citrate.

Concerning het, I had been planning for some time to carry out the same kind of experiments that you probably have in mind. The diploids are probably too unstable to give very much information on the simple determination of killing rates. Also, unfortunately, they seem to carry already a lethal deficiency which confuses matters. I have hoped, however, ~~the~~ to compare the rate at which lethal mutations (recessive) are accumulated with that at which haploid cells are killed, hoping to find some correction for multinucleate cells. I am sending you W-477, a T-L-B_1-V_1^r het stock, however, if you would like to make an attempt at quantitative studies of radiation killing of diploid cells. Lac+ prototrophs from W-477 x 58-161 should be picked from EMS lactose, and streaked out on EMB lactose. Suspensions corresponding to Lacv streaks can then be propagated by streaking out on EMS lactose, etc. After a few initial disappointments, you should have no trouble finding and maintaining the diploids, if you attend to them. May I assume that our studies in this direction will not interfere? If you really have a vital interest in this particular direction, however, I will withdraw. K. C. Atwood, at Columbia University has been carrying out comparable experiments on Neurospora conidia (heterokaryotic for a variety of markers) and concluded [according to an abstract in the Proceedings of the forthcoming Genetics Society meeting] that lethal mutations are not accumulated as rapidly as killings. so that they account for only part of radio-lethal effects.

*also Lac_1^-

Our cytological comparisons of diploid and haploid cells are progressing slowly. partly owing to the fact that we have not yet received apochromatic optics ordered some time ago. However, it seems quite clear that the typical diploid cell is quite distinct from the typical haploid, especially insofar as the nuclear structure can often be resolved into a number of smaller separate? bodies, which cannot usually be done with the haploids. Since there seems to be only a single chromosome however, I don't know yet how we will be able to interpret these bodies.

In summary, I am sending under separate cover the following stocks:

W-477 [K-12] T-~~L~~-B_1- Lac_1-mV_1^r het

W-1113 prototrophic, fertile coli from chickens, and its derivatives:

W-1205 threonine, histidine, lactose-, (produces prototrophs; very rough)

W-1189 valine; arginine+glutamic, maltose-

P.S. I don't think that het stocks are especially useful for proving allelism. If two Lac- factors are so closely linked that crossovers are very rare, then crosses on Lactose EMS will give occasional spontaneous heterozygous diploids anyhow. Crosses on EMS Lac are the best for Lac allelism. For phage tests, I would cross likewise, but test numerous colonies without bothering to purifying them from contaminating parents, instead making the tests on minimal or EMS medium.

Yours sincerely,

Joshua Lederberg